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EMBRYONAL MALIGNANCIES

Detection of the GD2⁺/CD56⁺/CD45[—] Immunophenotype by Flow Cytometry in Cerebrospinal Fluids from a Patient with Retinoblastoma

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Triple-color flow cytometry with a panel of antibodies comprising GD2, CD56, and CD45 was performed to analyze cerebrospinal fluids (CSF) from a patient with retinoblastoma who was suspicious of meningeal metastasis based on clinical presentation. Our results showed that the cells in CSF demonstrated the immunophenotype positive for GD2 and CD56 but negative for CD45 antigen, which suggested the presence of CSF metastasis of retinoblastoma. At the end of eight cycles of intrathecal chemotherapy, CSF specimen was analyzed with Flow cytometry immunophenotyping (FCI) again and the result showed no detectable malignant cells with the same immunophenotype. Our conclusion is that FCI can be a quick and reliable method for the diagnosis of CSF metastasis of retinoblastoma and the immunophenotype (GD2⁺, CD56⁺, and CD45[—]) can be used to recognize residual retinoblastoma cells in CSF.

Keywords cerebrospinal fluid, diagnosis, flow cytometry, metastasis, retinoblastoma

INTRODUCTION

Retinoblastoma is an intraocular tumor that mainly occurs in children. The occurrence rate is 1 in 20,000 in the Western population, with a relatively higher incidence in Chinese population [1, 2]. The treatment outcome of retinoblastoma has been dramatically improved in the past decades, with overall cure rates of >90% [3]. If the retinoblastoma extends beyond the globe, either by local extension or by distant metastasis, the use of chemotherapy, radiation, or even autologous stem cell transplantation rescue might be required [4]. Cerebrospinal fluid (CSF) metastasis is the most difficult type of retinoblastoma metastasis to cure and the early detection of the tumor cells in CSF is critical for proper and timely treatment. However, the precise diagnosis of retinoblastoma in CSF cytology is a challenge that requires ancillary investigations [5]. Flow cytometry immunophenotyping (FCI) is widely used for leukemia and lymphoma diagnosis, which has been shown to increase the overall diagnostic

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accuracy in many studies [6]. However, FCI analysis of CSF from retinoblastoma patients has not or rarely been reported. Some investigators have used aggressive bone marrow (BM) evaluation including immunocytology with ganglioside GD2 for advanced retinoblastoma [7]. Bryson et al. [8] reported that flow cytometric detection of the CD56+/CD45– immunophenotype is a useful rapid indicator for the presence of neuroendocrine malignancies. In this study, we conducted FCI analysis of the CSF from a child with retinoblastoma using a panel of antibodies comprising GD2, CD45, and CD56 and the result was reported as follows.

CASE REPORT AND FINDINGS

A 3-year-old male child was admitted to the emergency department of the Children's Hospital, Zhejiang University School of Medicine, with repeated twitching and altered consciousness for 1 day's duration without fever. Six months before he had been diagnosed as retinoblastoma in right eye by histological examination and performed enucleation of the right eye under general anesthesia. His complete blood count (CBC) revealed a WBC of $13.1 \times 10^9/L$ with 72.4% of neutrophils and a normal C-reaction protein (CRP). Computed tomography (CT) scan showed small increased density nodules in the edge of suprasellar region and of bilateral sylvian cistern and suggested that meningeal metastasis of retinoblastoma could not be excluded (Figure 1). Lumbar puncture was performed when this child was admitted, which revealed normal opening pressure. Routine analysis of CSF showed a cell count of $60 \times 10^6/L$ with 90% mononuclear cells and moderately high protein content. Smear examination of the pellet of CFS showed clumps of malignant cells. The CSF specimen was sent to Hematology-Oncology Laboratory for FCI analysis simultaneously. Immunophenotyping was performed by flow cytometer (FACSCalibur™; Becton Dickinson, San Jose, CA) with triple-color immunofluorescent staining using GD2-FITC, CD56-PE, and CD45-PerCP (BD Biosciences/Pharmingen, San Jose, CA). Data acquisition and analysis were performed using CellQuest software (Becton Dickinson) and Flowjo7.6 software (Tree star, Inc.), respectively. The FCI result showed that the cells in CSF demonstrated an immunophenotype with expressions



FIGURE 1 Computed tomography (CT) scan showed small increased density nodules in the edge of suprasellar region.

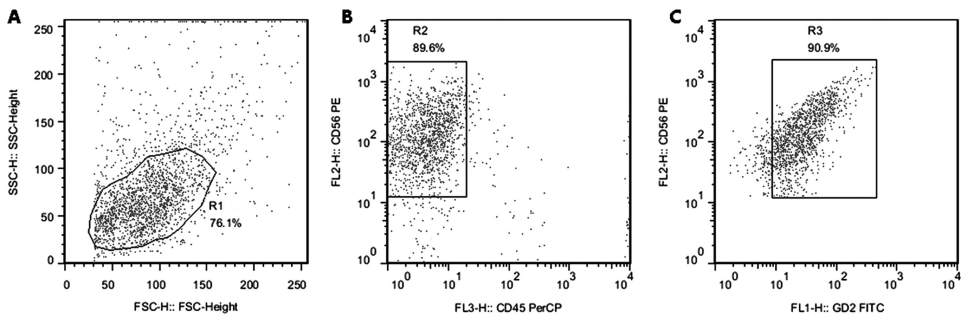


FIGURE 2 Triple-color flow cytometric analysis of a CSF specimen with retinoblastoma using GD2/CD56/CD45 MoAb combination. (A) Biparametric dot plot (R1 region) on forward scatter (FSC) and side scatter (SSC). (B) FCI diagram for detecting a population of cells with CD56+/CD45– immunophenotype in R2 region. (C) FCI diagram for demonstrating a population of cells with CD56+/GD2+/CD45– in R3 region. R2 gate refers to R1 and R3 refers to R2.

of GD2 and CD56 but no expression of CD45 antigen (Figure 2). This result confirmed the clinical suspicion of CSF metastasis of retinoblastoma. The child was referred to the pediatric oncology department where eight cycles of intrathecal chemotherapy (12.5 mg of methotrexate and 5 mg of dexamethasone) was administered. Craniospinal radiation was conducted as well. The patient’s condition improved significantly after combined modality therapy. At the end of eight cycles of intrathecal chemotherapy, lumbar puncture was performed and CSF specimen was analyzed with FCI again. The cells with GD2+CD56+CD45– immunophenotype were not found (Figure 3).

DISCUSSION

Previous study showed that spinal MRI or CT alone may miss some patients with CSF metastasis of tumor, and cytomorphological examination of CSF may allow an easy and quick identification of tumor cells [9]. However, the cytomorphological diagnosis can be difficult if the CSF involvement is early with too few tumor cells or if the tumor cells are small or different sized, as the tumor cells may be difficult to distinguish from lymphoid cells and other inflammatory cells. In addition, the presence of degenerative/apoptotic changes in some CSF specimens may also result in difficulty in recognizing tumor cells by cytology. Furthermore, the most unfavorable factor in the

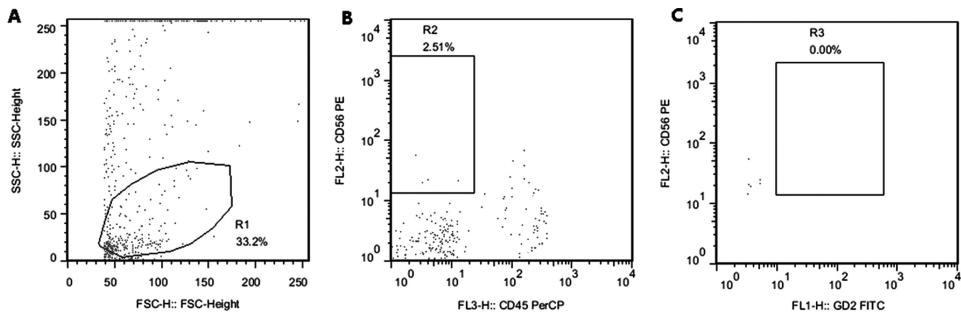


FIGURE 3 Triple-color flow cytometric analysis of a CSF specimen from the same patient with retinoblastoma after combined modality therapy. The cells with GD2+CD56+CD45– immunophenotype were not found. R2 gate refers to R1 and R3 refers to R2.

diagnosis of retinoblastoma with cytology in CSF is that the tumor cells do not form Flexner–Wintersteiner or Homer–Wright rosette, which is an evidence for retinoblastoma diagnosis in histopathology.

Flow cytometry is a very useful methodology in hematopathology and oncopathology. This technique provides rapid analysis of multiple characteristics of separate cell populations based on their sizes, cytoplasmic granularities, and antigens expressed. It can also assess co-expression of two or more antigens on the same cell population. It can be employed in almost all types of solid tissue specimens and body fluids. Recently, flow cytometry has been accepted as an adjunct to cytomorphologic diagnosis for neoplastic cells in serous cavity effusions. Cesana et al. [10] have shown that FCI displays a higher diagnostic performance than cytomorphological diagnosis in the analysis of serous cavity effusions (sensitivity, 96% vs. 60%). In a study by Czader and Ali [11], FCI modified the provisional cytomorphological diagnosis in 16% of 115 consecutive serous cavity effusions. Flow cytometry immunophenotyping is also recommended for detecting neoplastic cells in the evaluation of CSF specimens [12].

The utility of FCI in the diagnosis of retinoblastoma is very limited due to lack of specific markers. Previous studies showed that retinoblastoma originates from a primitive bipotential or multipotential neuroectodermal cell [13, 14]. Some neural cell adhesion antigens can be used to detect retinoblastoma cells. Some investigators have used aggressive bone marrow (BM) evaluation including immunocytology with ganglioside GD2 for advanced retinoblastoma [7]. Laurent et al. [15] have demonstrated the presence of GD2 synthase mRNA in blood and CSF samples in cases of extraocular dissemination of the RB tumor. CD56 is the cluster differentiation assignment for the neural cell adhesion molecule, a membrane glycoprotein containing immunoglobulin-like domains [16]. Natural Killer (NK) cells principally express the 140 kDa isoform of CD56. CD45 is a hematopoietic lineage-restricted cell-surface marker expressed on all hematopoietic cells, from HSCs to mature blood cells, except for erythroid cells. The recognition of CD56+/CD45– immunophenotype is important to avoid the potential misclassification of such specimens as NK cell malignancies. Bryson et al. [8] reported that flow cytometric detection of the CD56+/CD45– immunophenotype is a useful rapid indicator for the presence of neuroendocrine malignancies. However, no reports using the immunophenotype of GD2+/CD56+/CD45– for the diagnosis of CSF metastasis in patients with retinoblastoma with flow cytometry has been documented in the literature. In our study, GD2/CD56/CD45 tricolor combinations were tailored for detecting retinoblastoma. Our FCI results showed that retinoblastoma cells in CSF demonstrated an immunophenotype with GD2+/CD56+/CD45–. Although this immunophenotype is not retinoblastoma-specific, retinoblastoma cells in CSF can be detected by flow cytometry using these markers in patients with previous clinical diagnosis of retinoblastoma. This is a quick and reliable method for diagnosis of CSF metastasis of retinoblastoma. In addition, the FCI can be used to detect residual retinoblastoma cells in CSF. Due to the low occurrence rate of retinoblastoma in the populations and meningeal metastasis of retinoblastoma occurs much less frequently with an incidence of about 5% in patients with retinoblastoma [17, 18], we can only report one patient with FCI data. However, it still suggested that this method is useful for the diagnosis of CSF metastasis of retinoblastoma, especially when CSF cytology/MRI/CT fail to detect CNS metastatic disease.

To our knowledge, this is the first report for the diagnosis of CSF metastasis of retinoblastoma by flow cytometry. Our study shows that FCI analysis using a panel of antibodies comprising GD2, CD56, and CD45 is very helpful for the diagnosis of CSF metastasis in patients with retinoblastoma, which may be useful during the follow-up of patients with high risk of metastasis as well.

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Declaration of Interest

The authors declare no conflict of interests. The authors alone are responsible for the content and writing of the paper.

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